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Purported Mcl-1 inhibitor marinopyrrole A fails to show selective cytotoxicity for Mcl-1-dependent cell lines

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Dear Editor,

Anti-apoptotic Bcl-2 family proteins Bcl-2, Bcl-xL, and Mcl-1 have become prime targets for anti-cancer agents in an effort to overcome blocks in apoptosis exhibited by cancer cells.[1] Marinopyrrole A, a marine natural product originally developed for its activity against Methicillin-resistant *Staphylococcus aureus*,[2,3] was recently reported by Doi *et al.*[4] to be a selective Mcl-1 antagonist that binds to Mcl-1 and targets it for degradation. This publication was received with great interest because the discovery of a specific Mcl-1 inhibitor had thus far eluded researchers. Indeed, the only agents reported to target Mcl-1 to date are general transcription inhibitors, which show limited selectivity for Mcl-1 due to its short half-life.[5,6] However, previous studies in human colon carcinoma cells reported actin

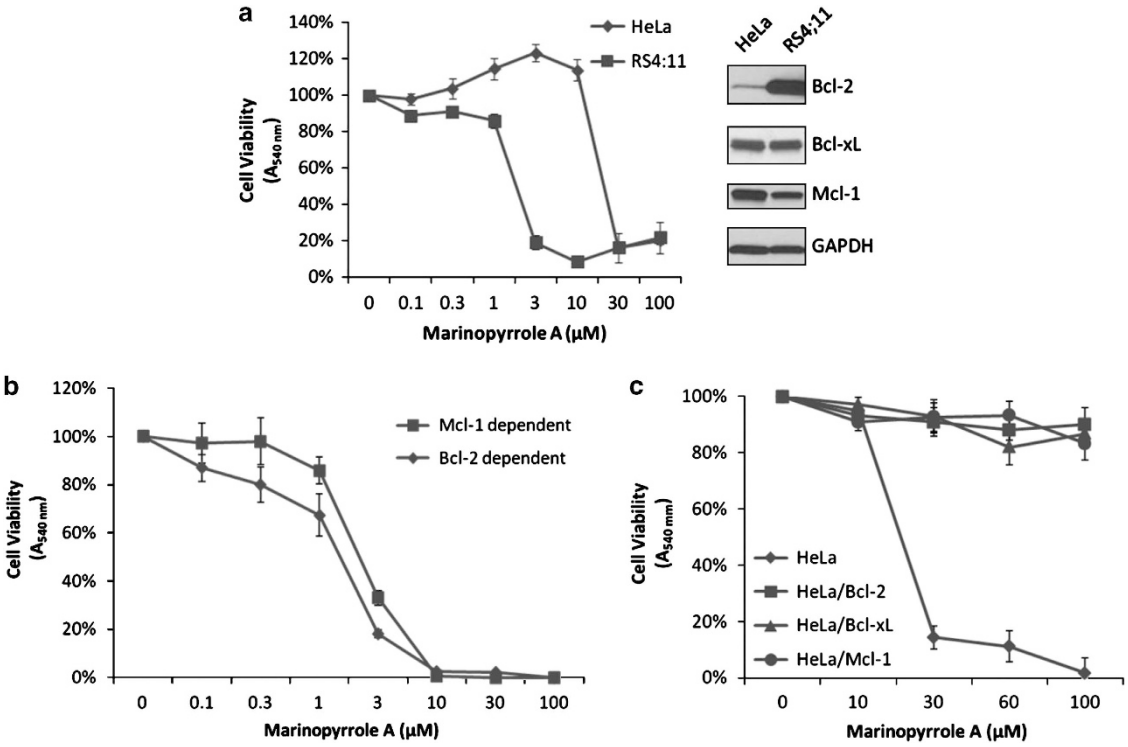

Figure 1 Marinopyrrole A is not selectively cytotoxic for Mcl-1-dependent cell lines. Cell viability was assessed using an MTT ((3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) cell viability assay. Cells were untreated (100%) or treated with marinopyrrole A (0.1–100 μM) for 72 h. Results given are mean ± s.d. ($n = 6$). (**a**) Left: cell viability curves for HeLa and RS4:11 (Bcl-2 dependent) cells. Right: immunoblot analysis of relative levels of anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1. GAPDH was used as a loading control. (**b**) Cell viability curves for Mcl-1-dependent and Bcl-2-dependent leukemia cells. (**c**) Cell viability curves for control HeLa cells and HeLa cells generated to stably overexpress untagged full-length Bcl-2, Bcl-xL, or Mcl-1. Cell extraction, immunoblotting, and cell viability assays using MTT dye were performed as described previously.[9] Antibodies against Bcl-2 (sc-509) and Mcl-1 (sc-819) were from Santa Cruz (Santa Cruz, CA) and antibodies against Bcl-xL (2762) and GAPDH (14C10) were from Cell Signaling (Beverly, MA)

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as the primary target of marinopyrrrole A.[2] This raised the possibility that Mcl-1 may not be the primary target for marinopyrrole A in cancer cells. We therefore sought to determine whether marinopyrrole A was indeed a *bona fide*-specific Mcl-1 inhibitor.

Our recent studies testing the viability of HeLa cells after systematic knockdown of individual anti-apoptotic Bcl-2 proteins demonstrated strict dependence on Mcl-1 (Eichhorn *et al.*, manuscript submitted). We first compared the sensitivity to marinopyrrole of Mcl-1-dependent HeLa cells *versus* RS4;11 human leukemia cells which have previously been shown to be dependent on Bcl-2 for survival.[7] HeLa and RS4;11 cells were treated with increasing concentrations of natural marinopyrrole A (0.1–100 μM) and cell viability assessed after 72 h (Figure 1a). Relative expression levels of Bcl-2, Bcl-xL, and Mcl-1 (Figure 1a, right) highlight Bcl-2 overexpression in the RS4;11 cell line. Surprisingly, marinopyrrole A was much more effective against Bcl-2-dependent RS4;11 cells (IC_{50}: 2 μM) when compared to Mcl-1-dependent HeLa cells (IC_{50}: 20 μM) (Figure 1a). Immunoblotting indicated that marinopyrrole A treatment did not affect Mcl-1 expression levels (data not shown).

We next tested whether natural marinopyrrole A would show selective cytotoxicity against Mcl-1- *versus* Bcl-2-dependent leukemia cell lines as reported by Doi *et al.*[4] For this purpose we chose two murine leukemia cell lines generated from transgenic mice and established via multiple criteria to be either dependent on Mcl-1 or Bcl-2.[8] Marinopyrrole A was equally effective against Mcl-1-dependent leukemia cells (IC_{50}: 2.5 μM) as Bcl-2-dependent cells (IC_{50}: 2 μM; Figure 1b).

It is important to note that data presented by Doi *et al.*[4] utilized a racemic mixture of synthetic marinopyrrole A, whereas our data and previous work done by Hughes *et al.*[2] were performed using the natural M-(-)-enantiomer. Therefore, cell viability assays were conducted in the Mcl-1- and Bcl-2-dependent leukemia cell lines using the non-natural P-(+)-enantiomer, and no statistical difference between the two enantiomers was observed (results not shown).

Finally, we tested whether overexpression of Bcl-2, Bcl-xL, or Mcl-1 would protect HeLa cells from marinopyrrole A. HeLa cells stably overexpressing Bcl-2, Bcl-xL, or Mcl-1 were treated with marinopyrrole A for 72 h and cell viability was assessed. Strikingly, overexpression of each anti-apoptotic Bcl-2 family member conferred complete resistance to marinopyrrole A (Figure 1c). Thus, marinopyrrole A failed to show selective cytotoxicity towards cells overexpressing Mcl-1 in contrast to previously reported data.[4] The data in Figure 1c indicate that the death signals generated from marinopyrrole A activate the intrinsic apoptotic pathway.

The results reported here are in stark contrast to previously reported data showing that marinopyrrole A induces cell death selectively in Mcl-1-dependent but not Bcl-2-dependent cells.[4] While further studies are needed to establish the basis of this discrepancy, taken together, our results seriously question the validity of marinopyrrole A as a specific Mcl-1 inhibitor.

Conflict of Interest

The authors declare no conflict of interest.

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